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Efficacy of Trovafloxacin for Treatment of Experimental *Bacteroides* Infection in Young and Senescent Mice

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We investigated the efficacy of trovafloxacin, a new quinolone, in comparison with that of clindamycin in the treatment of intra-abdominal abscesses caused by *Bacteroides fragilis* in young and senescent mice. The development of abscess formation, the number of viable organisms, and antibiotic concentrations were measured, and the values for young and old mice were compared. Trovafloxacin was well distributed to the tissues in both young and old animals. Although the pharmacokinetics and concentrations of trovafloxacin in serum were similar between young and old mice, the levels in tissue were higher in senescent mice than in young mice. Trovafloxacin therapy sterilized abscesses in 94% of young mice and in 73% of old mice, but this difference was not significant. This therapeutic response to trovafloxacin was similar to that seen with clindamycin. These results suggest that aging may not have any adverse effect on the therapeutic outcome for intra-abdominal abscesses caused by *B. fragilis*.

Infections are common in elderly people. Age-associated alterations in host immune cell functions may play a role in the occurrence of infections even with less virulent bacteria (8–10, 12). Aging is also associated with alterations in drug absorption, distribution, and elimination and with increased susceptibility to antibiotic toxicities (5, 7, 13, 14, 23). It is therefore important to evaluate the efficacies of antibiotics not only in young subjects but also in old subjects.

Intra-abdominal abscesses in elderly people causes high rates of mortality: The mortality rate in patients with subphrenic abscess is about 10% during the second decade of life, whereas it is about 60% during the seventh decade of life (1). *Bacteroides fragilis*, an anaerobic gram-negative bacillus, is a key pathogen in abdominal abscesses. Clindamycin is known to be effective in cases of abdominal abscess caused by *B. fragilis* and other anaerobes (19). In recent years, a variety of quinolones have emerged as effective antimicrobial agents against infections caused by *B. fragilis* and other anaerobes (2, 4, 20). Trovafloxacin, a new trifluoroquinolone undergoing clinical trials, has been shown to be effective against *B. fragilis* in vitro (4). In this study, we investigated abscess formation and compared the therapeutic efficacy of trovafloxacin to that of clindamycin in senescent and young mice.

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MATERIALS AND METHODS

Animals. Young (age, 2 to 3 months) and old (age, 19 to 22 months) male Swiss Webster mice were obtained from the National Institute of Aging, Bethesda, Md. All older animals were housed in separate cages. The animals were kept under conventional conditions.

Inoculum. The *B. fragilis* isolate used in this experiment was obtained from a patient with an intra-abdominal abscess and was identified by methods described by the Virginia Polytechnic Institute (11). The organism was passaged through the peritoneal cavities of mice three times to ensure its virulence, as evidenced by consistent production of an intra-abdominal abscess. The inoculum was prepared as described before (6). Briefly, the intra-abdominal abscesses in mice were induced by intraperitoneal injections of 0.1 ml of inoculum containing 10^7 CFU of *B. fragilis* with 50% autoclaved mouse fecal contents along with 10% barium sulfate.

Antibiotic therapy. A set of young mice and a set of senescent mice were assigned to three treatment groups. Therapy was initiated 24 h after intraperitoneal injection of the inoculum. The mice were treated with either clindamycin (commerically available) or alatrovafloxacin (CP-116,517-27), a prodrug of trovafloxacin (Pfizer Inc., Groton, Conn.). Trovafloxacin and clindamycin were given intraperitoneally three times a day at 4-h intervals. The dosages of clindamycin and trovafloxacin were 75 and 40 mg/kg of body weight/dose, respectively. Mice that were infected but not treated with any antibiotics were used as controls.

Quantitative analysis. Antibiotic therapy was given for 10 days. Two days after the discontinuation of antibiotics, all mice were sacrificed by cervical dislocation. At autopsy, abscesses were removed aseptically and weighed. The abscesses were homogenized with 1 ml of saline in ground glass homogenizers. Tenfold serial dilutions of the homogenate were made in saline, and 0.1 ml of each dilution was spread onto anaerobic brucella blood agar plates. This was done in triplicate. The plates were incubated anaerobically for 72 h at 37°C, and the numbers of CFU were counted at the end of the incubation. The animal was considered cured if the pus was sterile or there was no intraperitoneal abscess. The results were analyzed for statistical significance by the Z test for differences in proportions.

Trovafloxacin levels. Trovafloxacin levels in serum, pus, and tissue were determined for a set of 12 infected young mice and 10 infected old mice after 10 days of therapy. This was done to determine the concentration of trovafloxacin in infected animals after the administration of multiple doses of the antibiotic. On the 11th day, 1 h after administration of the first dose of antibiotic, a group of three old animals and three young animals were killed and blood samples were drawn for determination of serum antibiotic levels. Pus and tissue samples from the lung, liver, spleen, and small intestines were obtained. Intestinal debris was removed prior to determination of antibiotic levels in this tissue. Antibiotic levels were also determined 4, 24, and 48 h after administration of the first dose. All serum, pus, and tissue specimens were kept in dry ice and were mailed to Pfizer Central Research, Groton, Conn., for drug concentration determination. The assay for trovafloxacin was conducted by reverse-phase high-performance liquid chromatography as described by Girard et al. (4).

MIC. The MIC for the *B. fragilis* strain used in this study was determined by the broth microdilution method. Plates were incubated anaerobically at 37°C. The test isolate of *B. fragilis* used in this study was susceptible to both clindamycin (MIC, 1.5 μg/ml) and trovafloxacin (MIC, 0.24 μg/ml). The *B. fragilis* strain used for comparison, strain ATCC 25258, was also susceptible to clindamycin (MIC, 2.9 μg/ml) and trovafloxacin (MIC, 0.24 μg/ml).

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TABLE 1. Efficacy of antibiotic therapy in young and old mice with *B. fragilis*-associated intra-abdominal infection

Treatment	Age (mo)	No. of mice with positive abscesses/total no. of mice (% cure)	*B. fragilis* inoculum (CFU/mg)
Trovafloxacin	2–3	1/16 (94)[a]	1.0×10^3
	19–22	4/15 (73)[a]	3.1×10^4
Clindamycin	2–3	1/15 (93)[b]	1.0×10^6
	19–22	2/16 (88)[b]	8.5×10^5
Control	2–3	15/15 (0)	2.6×10^5
	19–22	13/16 (19)	4.2×10^5

[a] The cure rate for trovafloxacin-treated young mice was not significantly different from that for older mice (0.94 versus 0.73, respectively; Z = 1.1).
[b] The difference in the cure rate for clindamycin-treated young mice and older mice (0.93 versus 0.88, respectively; Z = 0.2) was not statistically significant.

RESULTS

Therapeutic efficacies of antibiotics. The results describing the therapeutic efficacies of the antibiotics are presented in Table 1. All 15 young mice used as infected controls (not treated with antibiotics) developed multiple abscesses, and *B. fragilis* was recovered from the abscesses. In contrast, 13 of 16 senescent mice developed multiple abscesses. At autopsy it was observed that the abscess size in senescent mice was considerably larger than that in the young mice. On clindamycin therapy, all but 1 of the 15 young mice were cured (93%), and among the old mice, 14 of 16 (88%) were cured. Trovafloxacin therapy cured 94% of the young mice and 73% of the old mice. This difference in cure rate with trovafloxacin between young and old mice was not statistically significant. There was no statistically significant difference between the cure rate for mice treated with clindamycin and that for mice treated with trovafloxacin. However, in mice treated with trovafloxacin there was a substantial reduction in the number of organisms recovered from the abscesses (Table 1).

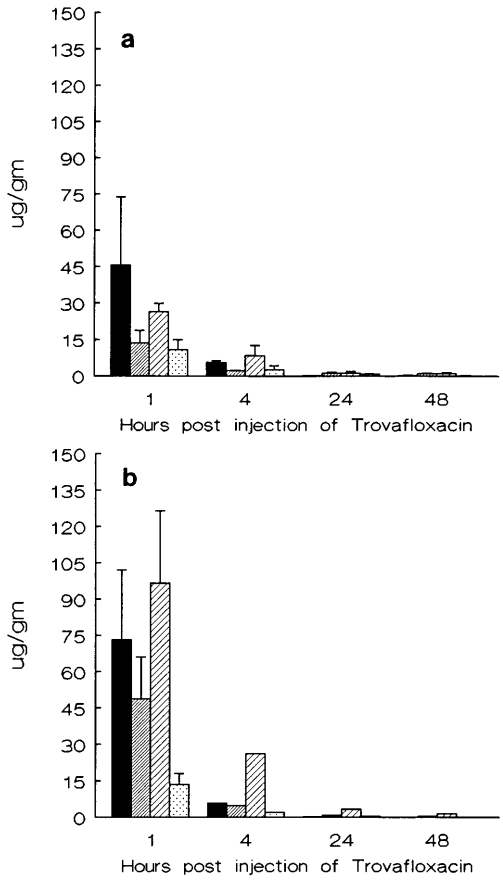

FIG. 1. Trovafloxacin levels in tissues of young (a) and old (b) mice. ■, liver; ▨, lung; ▨, spleen; ▨, intestine.

TABLE 2. Pharmacokinetics of trovafloxacin in mouse serum and tissue following injection of 40 mg/kg intraperitoneally[a]

Mouse group and tissue	C_{max} (μg/ml) or (μg/g)[b]	T_{max} (h)[c]	AUC (μg · h/ml) or (μg · h/g)	Tissue/serum ratio	
				C_{max}	AUC[d]
Young					
Liver	45.8	1.0	162.8	9.0	13.9
Lung	13.6	1.0	90.7	2.7	7.8
Spleen	26.5	1.0	192.6	5.2	16.5
Intestines	10.8	1.0	68.1	2.1	5.8
Abscess	27.1	1.0	204.6	5.3	17.5
Serum	5.1	1.0	11.7	1.0	1.0
Old					
Liver	73.3	1.0	214.3	12.6	17.3
Lung	48.8	1.0	176.4	8.4	14.2
Spleen	96.6	1.0	580.6	16.7	46.8
Intestines	13.4	1.0	51.7	2.3	4.2
Abscess	25.0	1.0	143.1	4.3	11.5
Serum	5.8	1.0	12.4	1.0	1.0

[a] Pharmacokinetic parameters were determined at 1, 4, 24, and 48 h after administration of the last dose. All mice received a dose of 40 mg/kg.
[b] Mean measured C_{max}.
[c] T_{max}, time to C_{max}.
[d] AUC from time zero to the last time point at which serum or tissue was obtained.

Trovafloxacin concentrations in serum and tissues. Following intraperitoneal injection of alatrovafloxacin (40 mg/kg/dose for 10 days) into the mice, trovafloxacin was well distributed in the liver, lung, spleen, intestines, and abscesses in both young and old animals (Fig. 1a and b and Table 2). The concentration and pharmacokinetics of trovafloxacin in serum were similar between both young and old mice (Fig. 2a and b and Table 2). In young mice, the mean serum trovafloxacin level at 1 h after injection was 5.1 ± 2.4 μg/ml, and it fell to 1.0 ± 0.2 μg/ml at 4 h. Trovafloxacin levels in serum were undetectable at 24 and 48 h. In old mice, the mean level was 5.8 ± 1.1 μg/ml at 1 h, which fell to 0.5 ± 0.5 μg/ml at 4 h. No drug was detectable at 24 and 48 h.

The mean antibiotic concentration in the abscesses of the young mice at 1 h was 27.1 ± 31.4 μg/mg, and it fell to a mean of 4.9 ± 2.5 μg/mg after 4 h. This was comparable to that in old mice at 1 h: 25 μg/mg, falling to 5.4 μg/mg. The measured exposure of the abscess to trovafloxacin, as assessed by the mean maximum concentration of drug (C_{max}) measured in serum, was similar between young and old animals. However, exposure of the abscess to trovafloxacin as assessed by the mean area under the concentration-time curve (AUC), was greater in younger animals due to a prolonged higher trovafloxacin concentration at this site at the later time points (24 and 48 h) in the younger animals. For all remaining tissues (lung, liver, and spleen), the mean trovafloxacin concentrations in tissue and the mean measured C_{max} and mean AUC were greater in older mice than in younger mice.

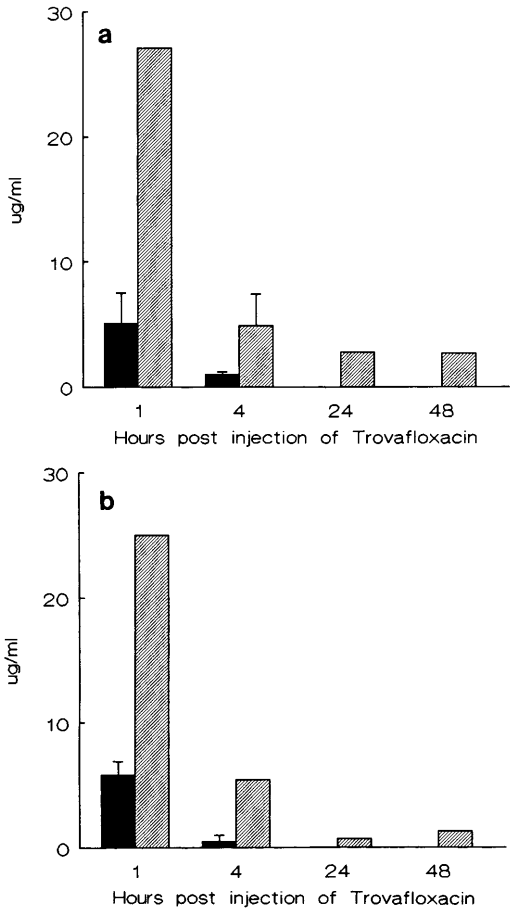

FIG. 2. Trovafloxacin levels in serum and abscesses of young (a) and old (b) mice. ■, serum; ▨, abscesses.

Clindamycin levels were not studied. In our earlier study we showed that subcutaneous administration of clindamycin (75 mg/kg/dose) resulted in levels in serum and pus of 5 and 2 μg/ml, respectively (6).

DISCUSSION

All intra-abdominal infections including intra-abdominal abscesses among elderly people carries a high mortality rate; presumably, it is due to atypical presentation of clinical features, delayed diagnosis, and delay in the initiation of treatment. It is known that in cases of intra-abdominal sepsis *Escherichia coli* and *B. fragilis* together play a key role in pathogenesis; *E. coli* is responsible for acute peritonitis and the sepsis that is associated with mortality, whereas *B. fragilis* is responsible for the formation of abscesses (21). The observation that the rate of mortality associated with *B. fragilis* abscesses in mice was low is consistent with the facts given above.

In our earlier studies with rats, trovafloxacin (CP99,219) was found to be effective against intra-abdominal abscesses caused by mixed infections with of *E. coli* and *B. fragilis*. In that study, we did not examine the efficacy of trovafloxacin in the elimination of infection in aged animals (20). The senescent mice used in this study are comparable to elderly human males ages 60 to 70 years. Such an age determination is arbitrarily based on expected mortality at a given age, i.e., 20% or less of the male Swiss Webster mice survive to 19 to 22 months of age, and about 1% or less live up to 32 to 36 months (2a, 9a). However, it should be mentioned that there is an immense diversity in the biology of aging in the animal kingdom, and therefore, one should exercise caution in extrapolating age-related findings for one species to another species.

Clindamycin is frequently used for the treatment of intra-abdominal abscesses due to *B. fragilis* (19). This antibiotic is expected to cure 88 to 93% of treated animals (2). Some quinolones like ciprofloxacin, ofloxacin, temafloxacin, PD117596, and PD127391 are known to be effective against *B. fragilis* infection (4, 11). Trovafloxacin, a new quinolone now undergoing clinical trials, has been found to be effective against *B. fragilis* in vitro (16). This study shows that trovafloxacin, like clindamycin, is effective against *B. fragilis* infection in both young and senescent mice. This would suggest that aging has no adverse effect on the efficacy of trovafloxacin in the treatment of *B. fragilis* infection.

The efficacy of trovafloxacin may be related to its distribution in serum and tissue. We showed that the levels and pharmacokinetics of trovafloxacin in the sera of young and old mice were similar. Our findings are in agreement with those presented in a recent report by Teng et al. (17). Those investigators showed that trovafloxacin pharmacokinetics are similar between young and aged subjects. In the senescent mice, trovafloxacin levels in the pus were five times higher than those in the serum. These levels were several magnitudes higher than the trovafloxacin MIC for *B. fragilis*. In addition, the spleens of the senescent mice had three to four times the levels of trovafloxacin found in the spleens of young mice. Quinolones in general are known to concentrate in the tissues, especially in the reticuloendothelial system (22). Trovafloxacin levels are higher in the tissues of the older animals, possibly due to the lipophilicity of quinolones. It is known that the level of body fat increases with age (15), and lipophilic antibiotics such as clindamycin, tetracyclines, and chloramphenicol are shown to achieve higher concentrations in tissues among the elderly (18).

In summary, our results demonstrate that trovafloxacin is as effective as clindamycin in treating *B. fragilis*-associated intra-abdominal abscesses in young and senescent mice.

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